REMARKS

With entry of the instant amendment claims 63 – 75 and 80 – 100 are pending in the application. Claims 69, 75 and 80 have been amended, and claims 83 – 100 are new. New matter has not been introduced by this amendment. Claims 15, 16, 18, 20 - 28, 30 – 51, 58 - 62 and 77 – 79 have been canceled. Applicants reserve the right to file further continuation applications on the subject matter of any canceled claim.

Claim 69 has been amended to correct the spelling of Enterobacteriaceae. Claim 75 has been amended to replace the abbreviation GDH with the term glucose dehydrogenase. The phrase "in any order" has been canceled from independent claim 80 and the phrase " by a 2,5-DKG reductase" has been included in clause d after, 2-KLG".

New claims 83 – 100 are dependent claims. Claims 83, 84, 85, 86, 87, 88, 89, 90, 91 and 92 correspond to now canceled claims 22, 23, 25, 26, 31, 47, 48, 49, 50 and 51 respectively. However, dependency has been changed. These claims now depend from independent claim 63. Claims 93 - 97 find support in now canceled claims 34, 37, 39, 41, 42 and 43. However, these claims now depend from independent claim 73. Claims 98 - 100 depend from independent claim 80. Claims 98 and 99 find support in original claims 11, 12 and 40, and claim 100 finds support in now canceled claim 79 and original claims 52 and 55.

The Examiner has objected to claims 37 and 69 because the term "Enterobacteriaceae" was been misspelled. Applicants have canceled claim 37 and corrected said error in claim 69.

Claims 15, 18, 20 - 28, 30 - 51 and 58 - 62 have been rejected under 35 U.S.C. §112, first paragraph for alleged lack of an adequate written description and for alleged lack of enablement. Claims 15, 18, 20 - 28, 30 - 51, 58 - 75 and 77 - 82 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite. Additionally, claims 15, 16, 18, 20, 24, 26, 27, 45 and 80 - 82 have been rejected under 35 U.S.C. §102(b) as being anticipated by Kulbe et al.; claims 15, 16, 18, 20 - 28, 30 - 51, 58 - 75 and 77 - 82 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Light et al., in view of Kulbe et al.; claims 15, 16, 18, 20 - 28, 30 - 51, 58 - 75 and 77 - 82 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Powers et al. in view of Kulbe et al.; claims 37, 39 - 41, 69, 70 and 75 have been rejected

under 35 U.S.C. §103(a) as being unpatentable over Light et al., in view of Kulbe et al. and further in view of Cha et al.; and claims 37, 39 - 41, 69, 70 and 75 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Powers et al., in view of Kulbe et al. and further in view of Cha et al. Applicants respectfully request the withdrawal of each rejection based on the arguments and amended claims presented herein.

Rejections under 35 U.S.C. §112, first paragraph.

The rejection of claims 15, 18, 20 - 28, 30 - 51 and 58 - 62 under 35 U.S.C. §112, first paragraph for alleged lack of an adequate written description and for alleged lack of enablement is moot in light of the cancellation of said claims.

Rejections under 35 U.S.C. §112, second paragraph.

The rejection of claims 15, 18, 20 - 28, 30 - 51 and 58 - 62 under 35 U.S.C. §112, second paragraph as being indefinite is rendered moot in light of the cancellation of said claims. Applicants assert claims 63 - 75 and 77 - 82 are clear and definite. Claim 80 has been amended to delete the phrase "in any order". However, Applicants are confused by the rejection of independent claim 63. While the Examiner has rejected claim 63 no explanation has been provided for said rejection.

Rejections under 35 U.S.C. §102(b).

The rejection of claims 15, 18, 20, 24, 26, 27 and 45 under 35 U.S.C. §102(b) as being anticipated by Kulbe et al. is rendered moot in light of the cancellation of said claims. With respect to claim 80 – 82, Kulbe et al., does not teach a process for the non-fermentative production of 2-KLG in an environment including host cells which comprise the recycling of the oxidized form of NAD^+ or $NADP^+$ and the reduced form of NADH or NADPH between and coupled to the oxidation of glucose by glucose dehydrogenase and the reduction step. For a reference to anticipate Applicants' claims each element must be taught in the prior art reference.

Rejections under 35 U.S.C. §103(a).

The rejection of claims 15, 16, 18, 20 - 28, 30 - 51, 58 – 62 under 35 U.S.C. §103(a) as being unpatentable over Light et al., in view of Kulbe et al. is rendered moot in light of the cancellation of said claims.

With respect to claims 63 - 75 and 77 - 82, Applicants assert the combination of Light et al. and Kulbe et al. do not render the claims unpatentable. As previously discussed Light et al. is concerned with an in vivo process. Additionally, Light et al. do not disclose a process for production of 2-KLG wherein at least one oxidative enzyme activity of the process requires an oxidized form of a co-factor and the reducing enzymatic activity of the process requires a reduced form of the co-factor wherein the reduced and oxidized co-factors are recycled between and coupled to said oxidizing step and reducing step. Recycling the oxidized and reduced form of the co-factor and the coupling of the reaction to the oxidization step and reduction step for regeneration is a critical element of the instant invention. This element is recited in each independent claim (claims 63, 73 and 80). At column 7, Light et al. discloses that the DKG reductase requires NADPH and that sources of electrons for the reduction of the coenzyme may be provided by any reduced substrate in contact with an enzyme for its oxidation, such as glucose/glucose dehydrogenase; glutamate/glutamate dehydrogenase; and formate/formate dehydrogenase. Further Light et al. disclose that other systems for regenerating NADPH cofactors are known in the art using, for example H_2 as a source of reducing equivalents and lipoamide dehydrogenase and hydrogenase or ferredoxin reductase and hydrogenase as catalysts. However, there is no teaching or suggestion that the process for the production of 2-KLG from a carbon source as disclosed in the instant specification could include a recycling and coupling of the co-factor between the oxidizing step and the reducing step such that co-factor is continually regenerated. One very significant advantage of the co-factor recycling of the claimed process is that enzymatic regeneration of the co-factor dependent reductase is not at the expense of another substrate that is oxidized. In the present invention, glucose can generate reducing power and still remain intact for conversion to 2KLG. This is not the case in the process as taught by Light et al. While glucose may be used for reducing power in Light et al., a portion of the glucose would breakdown to CO_2. Also as stated at page 11, lines 4 - 8 of the specification, this embodiment provides a means for co-factor regeneration, thereby eliminating the cost of continuously adding exogenous co-factor to the bioreactor for the production of KLG in Pantoea cells.

The combination of Kulbe et al. adds nothing to the already deficient disclose of Light et al. While Kulbe et al. may teach a process for intrasequential cofactor regeneration in enzymatic synthesis with one or a plurality of steps, and further that this process could be well adapted for

the production of ASA, the reference does not teach or suggest enzymatically oxidizing glucose by a glucose dehydrogenase to produce a first oxidation product, enzymatically oxidizing the first oxidation product to produce a second oxidation product, enzymatically oxidizing this second oxidation product to produce a third oxidation product; and enzymatically reducing the third oxidation product with 2,5-DKG reductase to form 2-KLG, wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor selected from NAD^+ or $NADP^+$ and the reductase requires a reduced form of the co-factor wherein the oxidized form and the reduced form are recycled between and coupled to the glucose oxidizing step and the reducing step. The Examiner has emphasized the abstract, Figures 4, 7 and 8 of Kulbe et al. as supporting the Examiner's position. However, Applicants submit these figures actually support the Applicant's contention that the reference does not teach the recycling and coupling of the co-factors as claimed in independent claims 63, 73 or 80. Even if this reference is combined with Light et al., Applicants have no idea what invention would be disclosed, but it clearly is not the instantly claimed invention.

The rejection of claims 15, 16, 18, 20 - 28, 30 - 51, 58 – 62 under 35 U.S.C. §103(a) as being unpatentable over Powers et al. in view of Kulbe et al. is rendered moot in light of the cancellation of said claims.

With respect to claims 63 - 75 and 77 - 82, Applicants assert the combination of Powers et al. and Kulbe et al. do not render the claims unpatentable. As previously discussed Powers et al. is concerned with the production of DKG mutants with improved properties. One mutant characterized by Powers et al., is the double mutant F22Y/A272G which is also used by the Applicants in the present application. There is no teaching or suggestion in the Powers et al. reference concerning a process for the production of KLG wherein the oxidized form and the reduced form of the required co-factor for the reducing step and at least one oxidization step are recycled between and coupled to said steps.

The combination of Kulbe et al. adds nothing to the already deficient disclose of Powers et al. As argued above, while Kulbe et al. may teach a process for intrasequential cofactor regeneration in enzymatic synthesis with one or a plurality of steps, and further that this process could be well adapted for the production of ASA, the reference does not teach or suggest enzymatically oxidizing glucose by a glucose dehydrogenase to produce a first oxidation product,

enzymatically oxidizing the first oxidation product to produce a second oxidation product, enzymatically oxidizing this second oxidation product to produce a third oxidation product; and enzymatically reducing the third oxidation product with 2,5-DKG reductase to form 2-KLG, wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor selected from NAD^+ or $NADP^+$ and the reductase requires a reduced form of the co-factor wherein the oxidized form and the reduced form are recycled between and coupled to the glucose oxidizing step and the reducing step. Even if this reference is combined with Powers et al., Applicants have no idea what invention would be taught, but it clearly is not the instantly claimed invention.

Applicants assert the cited references of Light et al., Powers et al., or Kulbe et al. alone or in any combination offer no suggestion or motivation to provide a process for the production of KLG wherein the process includes the recycling of co-factor between at least one oxidizing step and the reducing step of said process and without the waste of an added co-factor. Moreover beyond looking to the cited references to determine if it suggests doing what the inventors in this case have done, one must also consider if the cited references provide the required expectation of success. Both the suggestion and expectation of success must be founded in the cited references and not in Applicants' disclosure. In this case both the suggestion and the expectation of success are lacking.

The rejection of claims 37 and 39 - 41 under 35 U.S.C. §103(a) as being unpatentable over Light et al., in view of Kulbe et al. and further in view of Cha et al. is rendered moot in light of the cancellation of said claims.

Claims 69, 70 and 75 depend from independent claim 63. Claim 69 is directed to the process of claim 63 wherein the recombinant host cells are members of Enterobacteriaceae; claim 70 further defines the recombinant host cells as Pantoea cells and claim 75 further defines the process wherein a Pantoea cell is modified to eliminate the naturally occurring glucose dehydrogenase which oxidizes glucose to gluconate. Cha et al., has been cited for teaching an isolated Pantoea citrea gene encoding glucose dehydrogenase. While Cha et al., arguendo may disclose a Pantoea citrea gene encoding glucose dehydrogenase, the reference does not render the claims obvious. Applicants assert the independent claim, claim 63 is patentable over the cited references.

The rejection of claims 37 and 39 - 41 under 35 U.S.C. §103(a) as being unpatentable over Powers et al., in view of Kulbe et al. and further in view of Cha et al. is rendered moot in light of the cancellation of said claims. The same argument as provided in the preceding paragraph with respect to Cha et al., is repeated herein as applicable to claims 69, 70 and 75.

Applicants contend neither Kulbe et al., Light et al., Powers et al. nor Cha et al. taken alone or in combination render the claimed invention unpatentable. Moreover, Applicants contend a prima facie case of obviousness has not been supported by the Examiner. Withdrawal of all rejections under 35 U.S.C. §103(a) is requested.

In view of the foregoing, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (650) 846-7620.

Respectfully submitted,

Date: October 31, 2002

Lynn Marcus-Wyner
Registration No. 34,869

Genencor International, Inc.
925 Page Mill Road
Palo Alto, CA 94304
Tel: 650-846-7620
Fax: 650-845-6504

Enc: Marked-up Version of the Amended Claims
Clean copy of claims

MARKED-UP VERSION OF THE AMENDED CLAIMS

69. (Once amended) The process of Claim 68 wherein said recombinant host cells comprise members of **[Enterbacteriaces]** **Enterobacteriaceae**.

75. (Once amended) The process of Claim 74 wherein the host cell is obtained from a Pantoea species and the host cell is modified to eliminate the naturally occurring **[GDH activity]** **glucose dehydrogenase which oxidizes glucose to gluconate**.

80. (Once amended) A process for the non-fermentative production of 2-KLG in an environment comprising host cells, comprising the following steps **[in any order]**,

a. enzymatically oxidizing glucose by a glucose dehydrogenase to produce a first oxidation product, wherein said oxidation requires an oxidized form of an enzymatic co-factor;

b. enzymatically oxidizing said first oxidation product to produce a second oxidation product;

c. enzymatically oxidizing said second oxidation product to produce a third oxidation product; and

d. enzymatically reducing said third oxidation product to 2-KLG **by a 2,5-DKG reductase**, wherein said reduction requires a reduced form of said enzymatic co-factor
wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between and coupled to the first oxidizing step and the reducing step and said oxidized co-factor is NAD^+ or $NADP^+$ and said reduced co-factor is NADH or NADPH.

CLEAN CLAIM SET

15. (Canceled)

16. (Canceled)

18. (Canceled)

Claims 20 – 51 (Canceled)

Claims 58 – 62 (Canceled)

63. (Reiterated) A process for the non-fermentative production of 2-KLG from glucose comprising the following steps:

a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;

b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;

c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and

d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between and coupled to the glucose oxidizing step and the reducing step, and wherein the oxidized form of said co-factor is $NADP^+$ or NAD^+.

64. (Reiterated) The process of Claim 63 wherein the oxidized form of said co-factor is NAD^+ or $NADP^+$ and said reduced form of said co-factor is NADH or NADPH.

65. (Reiterated) The process of Claim 63 wherein said 2,5-DKG reductase is obtainable from a bacterial, yeast or fungal source.

66. (Reiterated) The process of Claim 63 that proceeds in an environment comprising exogenously added 2,5-DKG reductase.

67. (Reiterated) The process of Claim 63 wherein any one of the dehydrogenases are obtainable from a bacterial, yeast or fungal source.

68. (Reiterated) The process of Claim 63 that proceeds in an environment comprising recombinant host cells.

69. (Amended) The process of Claim 68 wherein said recombinant host cells comprise members of Enterobacteriaceae.

70. (Reiterated) The process of Claim 69 wherein said recombinant host cell is a Pantoea species.

71. (Reiterated) The process of Claim 68 wherein the host cell comprises a nucleic acid encoding a heterologous 2,5-DKG reductase.

72. (Reiterated) The process of Claim 68 wherein the host cell comprises a nucleic acid encoding a heterologous glucose dehydrogenase.

73. (Reiterated) A process for the non-fermentative production of 2-KLG from glucose comprising the following steps:

a. enzymatically oxidizing glucose by a glucose dehydrogenase to gluconate;
b. enzymatically oxidizing gluconate by a gluconic acid dehydrogenase to 2-KDG;
c. enzymatically oxidizing 2-KDG by a 2-KDG dehydrogenase to 2,5-DKG; and
d. enzymatically reducing 2,5-DKG by a 2,5-DKG reductase to 2-KLG

wherein the glucose dehydrogenase requires an oxidized form of an enzyme co-factor and said reductase requires a reduced form of said enzymatic co-factor and the oxidized co-factor and the reduced-cofactor are recycled between and coupled to the glucose oxidizing step and the reducing step, and

wherein the oxidized form of said co-factor is $NADP^+$ or NAD^+,

wherein the process proceeds in an environment wherein the 2,5-DKG reductase is provided exogenously to a host cell.

74. (Reiterated) The process of Claim 73 wherein the oxidized form of said co-factor is NAD^+ or $NADP^+$ and said reduced form of said co-factor is NADH or NADPH.

75. (Once amended) The process of Claim 74 wherein the host cell is obtained from a Pantoea species and the host cell is modified to eliminate the naturally occurring glucose dehydrogenase which oxidizes glucose to gluconate.

77. – 79 (Canceled).

80. (Once amended) A process for the non-fermentative production of 2-KLG in an environment comprising host cells, comprising the following steps,

a. enzymatically oxidizing glucose by a glucose dehydrogenase to produce a first oxidation product, wherein said oxidation requires an oxidized form of an enzymatic co-factor;

b. enzymatically oxidizing said first oxidation product to produce a second oxidation product;

c. enzymatically oxidizing said second oxidation product to produce a third oxidation product; and

d. enzymatically reducing said third oxidation product to 2-KLG by a 2,5-DKG reductase, wherein said reduction requires a reduced form of said enzymatic co-factor

wherein the oxidized form of said co-factor and the reduced form of said co-factor are recycled between and coupled to the first oxidizing step and the reducing step and said oxidized co-factor is NAD^+ or $NADP^+$ and said reduced co-factor is NADH or NADPH.

81.(Reiterated) The process of Claim 80 wherein the oxidized co-factor is NAD^+ and the reduced co-factor is NADH.

82.(Reiterated) The process of Claim 80 wherein the oxidized co-factor is $NADP^+$ and the reduced co-factor is NADPH.

83. (New) The process of Claim 63 wherein said glucose dehydrogenase activity is obtained from a bacterial, yeast or fungal source.

84. (New) The process of Claim 63 wherein said glucose dehydrogenase activity is obtained from a T. acidophilum, a Cryptococcus uniguttalatus or a Bacillus species.

85. (New) The process of Claim 63 wherein at least one of the enzymes of steps a, b, c or d is immobilized.

86. (New) The process of Claim 63 wherein at least one of the enzymes of steps a, b, c or d is in solution.

87. (New) The process of Claim 63 wherein the 2, 5-DKG reductase is obtained from Corynebacterium or Erwinia.

88. (New) The process of Claim 63 that is continuous.

89. (New) The process of Claim 63 that is batch.

90. (New) The process of Claim 63 that proceeds in an environment comprising organic solvents.

91. (New) The process of Claim 63 that proceeds in an environment comprising long polymers.

92. (New) The process of Claim 63 further comprising the step of obtaining ascorbic acid (ASA) from said 2-KLG.

93. (New) The process of Claim 73 that proceeds in an environment comprising recombinant host cells.

94. (New) The process of Claim 93 wherein said recombinant host cells comprise a member of Enterobacteriaceae.

95. (New) The process of Claim 93 wherein said recombinant host cells are Pantoea citrea cells.

96. (New) The process of Claim 93 wherein said recombinant host cells have a mutation in a membrane bound glucose dehydrogenase.

97. (New) The process of Claim 93 wherein said host cells further comprise nucleic acid encoding a heterologous glucose dehydrogenase.

98. (New) The process of claim 80 wherein the host cells comprise a member of Enterobacteriaceae.

99. (New) The process of Claim 98 wherein said recombinant host cells are Pantoea citrea cells.

100. (New) The process of Claim 80 wherein the host cells are modified to eliminate the naturally occurring glucose dehydrogenase which oxidizes glucose to gluconate and wherein the host cells are modified to include a heterologous glucose dehydrogenase having a specificity for NAD^+ or $NADP^+$.